Regeneration in Teleosts.

By

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With 14 figures in text.

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An examination, that I made, of fish that had just been caught and brought into the laboratory at Woods Holl showed that the tail had often been injured and had in many cases begun to regenerate. In discussing the question with Prof. H. C. BUMPUS he told me that he had experimented with *Fundulus* and had found that the tail readily regenerates. I began, therefore, my experiments on this form

Fig. 1.

Fig. 2.

(*Fundulus heteroclitus*). Cutting off the tail squarely at different levels I saw in a few days the formation of new material along the edge. This new part grew rapidly, and in the course of two weeks or longer, according to the amount removed, the tail regained its former size.

At this time I was studying the regeneration of planarians, and was greatly interested in regeneration from an obliquely cut surface.

I therefore tried the same experiment with the tail of *Fundulus*. The tail was cut off obliquely in several fish as shown in Fig. 1 (July 25). At the same time the tails of three other fish were cut

Fig. 3.

Fig. 4.

off by two oblique cuts as shown in Fig. 2; and of three others by two oblique cuts as shown in Fig. 3. In three other fish the tail was first split longitudinally, and then the lower half cut off by an

Fig. 1 a.

Fig. 2 a.

oblique cut, Fig. 4. Twelve days after the operation the fish were killed and one tail from each of the first three experiments is drawn¹⁾ in Figs. 1 a, 2 a, 3 a. In the first series, Fig. 1 a, the new material is broader at the lower part of the cut-edge than at the upper. In the second series, Fig. 2 a, the new material is broadest at the upper end of the upper oblique cut,

Fig. 3 a.

¹⁾ The figures that indicate the kind of operation, as Figs. 1, 2, 3 for example, are intended to represent the position of the cut on the living tail. The figures that represent the new, regenerated part, as Figs. 1 a, 2 a, 3 a for

and at the lowest end of the lower cut. In this case also the greatest amount of new material has developed at that part of the edge that is nearer the base of the tail. In the third series, Fig. 3 *a*, the new material is widest at the inner angle, where the oblique-cuts meet, i. e., again on that part of the oblique surface that lies nearer to the base of the tail. In the fourth series new material had developed at the exposed edge of the oblique part of the cut, but almost no new material was present along the side of the longitudinal cut.

Meanwhile another experiment had been started. The tail of one fish was cut squarely across at about the middle, Fig. 5, and

Fig. 5.

Fig. 5 *A*. Fig. 5 *a*. Fig. 5 *A*, *a*.

the tail of another was cut as shown in Fig. 5 *A*. In the former the new part, after nine days was broader at the middle than at the sides, Fig. 5 *a*. In other words it has assumed the characteristic, rounded form of the tail of *Fundulus*, although the new part had by no means replaced as much as had been lost. In this experiment the cut was made strictly across the tail, yet after a time the middle

example, are camera drawings of preserved material. The differences in size of the two kinds of drawings are in part the result of shrinkage, due to the preservatives in the latter series, and also due to fish of different sizes being used.

part grew faster than the sides, and as a result the new part assumed a rounded form. In the other experiment the new material grew faster at the inner part of the oblique surface and very little new material formed at the edge of the longitudinal cut, Fig. 5 *A, a*.

The more rapid growth that took place in all these experiments was in that part of the oblique cut that lies nearer the base of the tail. The results suggested that the new part might receive better nourishment at the base of the tail than further out, hence its more rapid growth. This view would also account for the graded rate of

Fig. 6 *a*. Fig. 6 *b*. Fig. 6 *c*. Fig. 6 *d*. Fig. 6 *e*.

growth from the inner to the outer part of the oblique cut. I attempted to test this interpretation of the process by cutting off the tail in some fish near the base, and in others near the outer part of the tail. The fish were kept under similar conditions, and the relative rate of growth examined from day to day. In an experiment begun Aug. 2 the tails of two large fish were cut-off, one near the outer end, and one near the base. At the same time the same operation was carried out on two smaller fish. Seven days later the amount of new material in each seemed to be about the same. Four days

later there was no more new tissue on the shorter tail than on the longer one. The experiment was repeated on Aug. 19, when one tail

Fig. 7.

Fig. 8.

Fig. 9.

was cut off near the base, one near the middle, and one near the outer part of the tail (the latter a little obliquely). The fish were killed on Aug. 25. The amount of new tissue is not obviously greater in the shorter, Fig. 6 *c*, than in the median tail, Fig. 6 *b*, or in the longer one, Fig. 6 *a*. The tails of two other fish were cut off obliquely at the same time. One had only the lower angle of the tail cut off, Fig. 6 *e*; the other had the entire tail cut across obliquely, Fig. 6 *d*. Six days later the tails appeared as drawn in Figs. 6 *e* and 6 *d*. The amount of new material at the base is about the same as at the maximum points in the tails cut across, Fig. 6 *a*, *b*, *c*; but nearer the outer parts of the tail the amount of new tissue is distinctly less than in Figs. 6 *a* and 6 *b*, that are cross-sections at corresponding levels. This comparison shows that the view that the more rapid growth is only due to the level at which the cut is made is insufficient to explain the phenomena.

The results of the experiment on tails cut obliquely may be stated in a teleological way as follows: The more rapid growth takes place in those regions where more new tissue is needed to complete the

entire structure. The general bearing of this statement will be further discussed later.

In another way I attempted to determine the meaning of the relation of the amount of growth from oblique surfaces. The tails

of two fish were cut squarely off as shown in Figs. 7 and 8; then one of these, Fig. 8, was cut off obliquely. The upper edge of the oblique cut (Fig. 8) lies at the same level as the cross-cut of the other tail (Fig. 7). The tail of a third fish, Fig. 9, was cut squarely off at its base, — at the level of the inner edge, *y*, of the oblique cut (Fig. 8). If the rate of growth over the oblique surface is determined by the nearness of the cut-surface at each point to the base

Fig. 7 *a*. Fig. 8 *a*. Fig. 9 *a*.

of the tail, then we should expect the new tissue at *x* in Fig. 8 to grow as fast as that over the squarely cut-surface of Fig. 7; and we should expect the growth at *y* in Fig. 8 to be the same as that over the cut-surface of Fig. 9. After eight days the fish were killed and their tails were drawn (Figs. 7 *a*, 8 *a*, 9 *a*). Comparing the rate of growth in the obliquely-cut tail with the other two we see at a glance it is narrower at the upper part than the broadest part of the

Fig. 7 *a'*. Fig. 8 *a'*. Fig. 9 *a'*.

new tissue in the tail cut across (Fig. 7), and that the growth at the lower edge, *y*, is even greater than that in the tail cut across at the same level (Fig. 9 *a*). In another experiment of the same sort on somewhat larger fish the results two days later are represented in Figs. 7 *a'*, 8 *a'*, 9 *a'*. Exactly the same results were obtained except that the growth at *y* in Fig. 8 *a'* is more nearly like that in the shorter tail, Fig. 9 *a'*. The difference in this regard that was found in the preceding experiment is probably due to the fact, that in that experiment the shorter tail (Fig. 9 *a*), was cut too near the base;

passing, in fact, through the scales at the base of the tail. The latter experiment is, therefore, more satisfactory.

These results show, I think, conclusively, that the more rapid growth at the inner part of the cut-edge in an oblique cut can not be explained by the nearness of the cut to the base of the tail as compared with the outer parts of the same cut-edge. The experiments also show that it is not so much the more rapid growth at y as the retardation of the growth at x that is in need of explanation; for the latter, at x , is less than that at the edge of a cross-section at the same level, while the growth at y is about the same as that at the same level in a cross-section.

Fig. 10.

Fig. 10 A.

Fig. 10 B.

It seemed to me that this problem of the rate of growth might be made clearer, if, instead of using a form with a rounded tail like *Fundulus*, I should use a fish with a swallow-tail. Through the courtesy of Prof. H. C. BUMPUS, in charge of the U. S. Fish Commission Laboratory at Woods Holl I was able to experiment with some of the fish in the aquaria of the station. I used first some young scup (*Stenopus chrysops*). The tail of this fish is distinctly bi-lobed, Fig. 10. In the first experiment (Aug. 9) the tails of five fish were cut squarely off near the base, Fig. 10 $a-a$; and the tails of five others were cut off obliquely as shown in Fig. 10 $b-b$. Seven days later new tissue was present along the cut-edge of all the tails, and it seemed at this time to be equally broad along its entire length. Two days later one could see that in the tails cut squarely off the outer parts were a little broader than the middle part, Fig. 10 A; and in the tails cut off obliquely the lower side of the new part was a little broader than the upper part, Fig. 10 B. In other words the new part was assuming the form of the old tail, although not as much new material had as yet developed as had been removed.

The most important fact shown by this experiment is that the regions of more rapid growth are directly connected with the development of the typical form of the tail, and not with the level at which the section has been made.

I repeated the experiment on some half-grown mackerel scad (*Decapterus macrelle*). The tail of this fish is more bifid (Fig. 11)

Fig. 11.

Fig. 11 A.

Fig. 11 A'.

than that of the scup. Four days after the operation the new material over the cut-edge was the same breadth along the entire edge, and it remained so for three days more, and then it began to grow faster at the upper and the lower parts. The day following it was distinctly bifid, Fig. 11 A, and continued to become more and more so (Fig. 11 A', four days older).

The tail of the king-fish (*Menticirrhus saxatilis*) has the form shown in Fig. 12. The tails of two young fish were cut off by a cross-cut as shown in Fig. 12. Seven days later the new material was equally well developed along the entire cut-edge in one of the two individuals; and in the other the new material was a little broader

Fig. 12.

over the lower side. Four days later this fish showed a distinctly longer part on the lower side; the other tail had grown irregularly, due perhaps to injury. The same experiment repeated on another individual showed also after a time more rapid growth on the lower side.

The results of these experiments on the scup and the mackerel scad fall into line with those on *Fundulus*, and show that the more

rapid growth takes place where more tissue is to be formed (teleological view), or better, the new material assumes the form of the old part before the new material has reached its full growth. The development of the typical form is brought about by more rapid growth taking place in one (or more) parts than in another.

Oblique position of the new part on an oblique surface.

The new fin-rays can be seen at a very early stage in the new part, and on closer examination it will be found that in tails cut off obliquely the new rays make an angle with the old ones as a result of their position at right angles to the cut-surface. In Fig. 13 the

Fig. 13.

Fig. 14.

position of the new rays is shown in a tail cut off by one oblique-cut. In Fig. 14 the new rays in both the upper and lower new portions make an angle with the old ones. When the tail is cut as in Fig. 2 we would expect to find the rays standing at an angle with the old ones but turning inwards in this case, I have not been able to convince myself entirely that they do so. After cross-section of the tail the new rays continue in the same line with the old ones.

There can be no doubt that the position of the new rays, in tails cut obliquely, at right-angles to the cut-surface, is the same phenomenon that has been observed in several other forms. BARFURTH¹⁾ in 1891 showed that if the tail of a tadpole is cut off by an oblique cut the new tail will stand at right-angles to the cut-surface. He also showed that in later stages the tail swings back into place. BARFURTH attempted to show by a series of ingenious experiments that this righting of the tail is a result of its being

¹⁾ BARFURTH, D., Versuche zur funktionellen Anpassung. Archiv f. mikr. Anat. 37. '91.

used as a swimming organ; for if the tadpoles were kept in shallow water, where they could not use their tails, the new tail did not in most cases swing back into line. The demonstration is not altogether satisfactory, as BARFURTH himself pointed out, since some of the tails did become straight under the conditions just described. In addition to the use of the tail as a factor in its straightening BARFURTH introduces other factors, »regulatorische Fähigkeiten«.

In 1895 I showed¹⁾ that in the earthworm the new head that develops from an oblique (lateral) surface stands at right-angles to the cut-surface. HESCHELER²⁾ ('96) independently obtained the same result. DRIESCH³⁾ has shown ('96) in *Tubularia* that the new tentacles that are laid down in the old part form an oblique row if the piece has been cut off obliquely. Whether this phenomenon belongs in the same category as the other cases described above may be open to question. KING⁴⁾ has shown that in the regeneration of an arm of the star-fish from an oblique surface the new arm stands at right-angles to the cut-surface.

In planarians I have shown⁵⁾ that when the anterior end is removed by an oblique-cut the new head, developing on the posterior piece, stands at an angle to the cut-surface. In these animals the new part appears not only at right-angles to the cut-surface, but it lies on only one side of the oblique-edge. In all the other forms described above the new part occupies the entire cut-surface. In these same planarians the new tail that develops on an oblique surface also stands at right-angles to that surface. The way in which the new head develops in planarians has given me a clue, I believe, to the interpretation of the phenomenon. The new head forms out of the new material long before it has increased sufficiently in amount to represent as much as was removed. The new head forms symmetrically in the new material, and since at this time the material has developed at about the same rate along the entire oblique surface, or at least in the region where the head appears, the median

¹⁾ MORGAN, T. H., A Study of Metamerism. J. J. M. S. 37. '95.

²⁾ HESCHELER, K., Über Regenerationsvorgänge bei Lumbriciden. Jena-ische Zeitschr. '96.

³⁾ DRIESCH, H., Zur Analyse der Reparationsbedingungen bei *Tubularia*. Vierteljahrsschr. d. Nat. Ges. Zürich. '96.

⁴⁾ KING, H. D., Regeneration in *Asterias vulgaris*. Archiv f. Entwicklungsmechanik. '98.

⁵⁾ MORGAN, T. H., Experimental Studies of the Regeneration of *Planaria maculata*. Archiv f. Entwicklungsmech. '98.

line of the new head must stand at right-angles to the surface, if a symmetrical head is to form in this material. The objection that may be made to this statement is to the assumption that a new head must form symmetrically in the new part. Why this should happen I can not, of course, pretend to say, but the statement rests on the observed fact that the new head does form in this way. As I have tried to show in another paper the new material along the cut-edge in these planarians may be thought of as totipotent, or at least a new head may develop at any point along the obliquely cut surface. The factor that determines the position of the new head is the most anterior part of the old tissue. The position of the head being fixed the new head forms symmetrically in the new material that has appeared, and, in consequence, stands at right-angles to the cut-surface.

The development of a new head at right-angles to the cut-surface is shown in its most extreme form in pieces of the planarian cut longitudinally from the side¹⁾. The new head stands, at first, at right-angles to the cut-surface, and therefore at right-angles to the long axis of the old part. The new head soon grows faster on one side, and in consequence swings around into an anterior position.

Can we apply this same interpretation to the regeneration from an oblique surface in the tail of the fish?

The simplest case is that of regeneration from one oblique cut (Figs. 1 and 13). My observations go to show that at first the amount of new material along the cut-edge is equal at every point. From this new material a tail may now be thought of as forming with the middle axis of the new tail in the middle of the new material. It will stand, therefore, at right-angles to the cut-surface, and make an angle with the middle ray of the old tail. The end of the middle ray of the old tail will determine the point at the base of the new tail from which the middle ray is to develop, and the position of the new middle ray may be determined by joining the end of the middle ray of the old tail with the outer symmetrical, middle point of the new tissue. The new tail stands, therefore, at first at right-angles to the old one, because it is at the beginning a symmetrical structure.

If this analysis is correct how can we account for the subsequent changes that bring about a more rapid development on one side, so

¹⁾ As shown by Fig. 7 in my paper *Regeneration in Planarians*. 1900.

that the new tail swings into line with the old one? It is obviously the same phenomenon by means of which the new head of the planarian is carried around into line with the long axis of the old part. The change is due to a more rapid growth on one side, but the factors that bring this about are entirely unknown. The problem is to account for more rapid, or for continuous growth on one side of a symmetrical structure by means of which the new part becomes itself unsymmetrical, but completes an unsymmetrical old part in such a way, that in the end the old and the new supplement each other to form a symmetrical whole. The factors producing these change, while exciting our highest interest, escape, at present, completely our power of analysis; unless we choose to adopt a vitalistic, teleological view of the problem. It is better, I think, to wait, in the hope that the discovery of further facts may make the interpretation possible.

It remains to be shown that the same interpretation can be applied to those cases in which only a part of the tail has been removed by one or by two oblique cuts, Figs. 2 and 14. The new part is here also at first formed symmetrically in the new material, that is, the middle ray of the series exposed at the cut-edge joined by an imaginary line to the symmetrical middle point of the free edge gives the position of the middle ray of the new part that stands, therefore, at right-angles to the cut-surface. The interpretation in this, as in the preceding case, goes no further than to assume that a symmetrical part is first laid down as observations verify. The causal explanation of this phenomenon is unknown to us at present.

Regeneration of the Fins of Fishes.

WEISMANN in his book on the Germ-plasm states that »fishes are unable to regenerate a lost pectoral or pelvic fin, while the much more highly organised Salamander has been known to regenerate a limb six times in succession«. I examined the power of regeneration in the pectoral and pelvic fins of *Ctenolabrus* with the following results. The pectoral fin was cut off at about its middle, and it regenerated completely. A pelvic fin had also partially regenerated when the fish was killed. The median post-anal fin also regenerated. The dorsal fin was in part removed, and had begun to regenerate when the experiment was brought to a close. The results show that all the fins in *Ctenolabrus* have the power of regeneration.

I tried another experiment with the pectoral fins of young individuals of *Batrachus tau*. Regeneration took place very slowly yet a new part was formed. The fish died before I could determine whether or not the fins would have regenerated fully.

The experiments described in the preceding pages show that regeneration of the caudal fin takes place in several genera of teleosts. How generally it occurs in the group must be determined by further experiments.

Summary.

1. If the tail of *Fundulus* is cut off at any level a new tail regenerates.

2. If the tail is cut off obliquely as shown in Fig. 1 the new tissue forms more rapidly at the part nearer the base of the tail, Fig. 1 *a*. If the tail is cut off by two oblique cuts as shown in Fig. 2 the new tissue grows more rapidly at two points, viz., those that lie nearer the base of the tail. If the tail is cut off by two oblique cuts, as shown in Fig. 3 the new tissue grows faster in the angle. In all cases, therefore, the more rapid growth takes place nearer the base of the tail.

In order to show that the faster growth is not due directly to its nearness of the region to the base of the tail the following experiment was made. The tail of one fish was cut off at its base, Fig. 6 *c*, of another at the middle, Fig. 6 *b*, of a third at the outer end, Fig. 6 *a*, and the rate of growth compared with that of a fish whose tail was cut off, at the same time, obliquely, Fig. 6 *d*. The new tissue in the tail cut across grows equally fast in all three cases. In the tail cut obliquely the part near the base grows at about the same rate as in the tails cut across, but near the outer part the growth is less rapid.

3. If the tail of *Stenopus* is cut squarely off, Fig. 10 *A*. The new tail, grows faster at two points and produces as a result the typical swallow-tail. If the tail is cut off obliquely, Fig. 10 *B*, the new tissue grows faster at the base where more is needed (teleological view).

4. The tail of *Decapterus* is more deeply bifid, Fig. 11, and gives similar results, Fig. 11 *A, A'*. The tail of *Menticirrhus* has a ventral lobe, Fig. 12. If the tail is cut squarely off it grows faster on the lower side.

The interpretation of the result seems to be that the new material assumes the typical form before it has reached its full size (causal view).

5. When the tail is cut off obliquely the rays in the new part stand at first at right-angles to the cut-surface. A similar result has been observed in several other animals. The interpretation of this relation is discussed in the text, and the conclusion reached, that it is due to the development of a symmetrical structure in the new part when differentiation first begins.

6. The pectoral and pelvic, dorsal, caudal and anal fins of *Fundulus* have the power of regeneration.

Bryn Mawr, Penn., U. S. A., Apr. 16 1900.

Zusammenfassung.

1. Wenn der Schwanz von *Fundulus* in irgend einem Höhenniveau abgetrennt wird, so erzeugt sich ein neuer Schwanz.

2. Schneidet man den Schwanz (nach Fig. 1) schräg ab, so entsteht das neue Gewebe rascher an dem Theil, der der Schwanzbasis näher liegt (Fig. 1 *a*). Wird der Schwanz durch zwei Schrägschnitte, wie in Fig. 2, abgetrennt, so wächst die Neubildung an zwei Punkten rascher wie an denjenigen, welche der Schwanzbasis näher liegen. Liegen die Schrägschnitte wie in Fig. 3, so wächst das neue Gewebe rascher in dem Winkel. In allen Fällen findet daher das Wachstum am schnellsten an den der Schwanzbasis nächst gelegenen Stellen statt.

Um zu zeigen, dass das schnellere Wachstum nicht unmittelbar durch die größere Nähe der Schwanzbasis bedingt ist, wurde folgender Versuch angestellt: der Schwanz eines Fisches wurde an seiner Basis abgeschnitten (Fig. 6 *c*), der eines anderen in der Mitte (Fig. 6 *b*), der eines dritten am distalen Ende (Fig. 6 *a*) und das Quantum des Zuwachses verglichen mit dem eines Fisches, dessen Schwanz zu gleicher Zeit schräg abgeschnitten worden war, Fig. 6 *d*. Das neue Gewebe wuchs in den drei quer abgeschnittenen Fällen gleich rasch an. Mit ihnen etwa gleich schnell wuchs der nächst der Schwanzbasis gelegene Theil des schräg abgeschnittenen Schwanzes; näher dem distalen Ende geht das Wachstum weniger schnell vor sich.

3. Wird der Schwanz von *Stenopus* quer abgeschnitten (Fig. 10 *A*), so wächst der neue Schwanz an zwei Punkten schneller und bildet so den typischen Schwalbenschwanz. Wird der Schwanz schräg abgeschnitten (Fig. 10 *B*), so wächst das neue Gewebe schneller an der Basis, wo mehr davon benüthigt wird (teleologischer Gesichtspunkt).

4. Der Schwanz von *Decapterus* ist tiefer zweigespalten (Fig. 11) und giebt ähnliche Resultate (Fig. 11 *A, A'*). Der Schwanz von *Menticirrhus* hat einen ventralen Lappen (Fig. 12). Schneidet man ihn quer ab, so wächst er auf der unteren Seite schneller.

Die (richtige) Ausdeutung des Resultats scheint die zu sein, dass das neue Material bereits die typische Form anzunehmen sucht, bevor es seine endgültige Größe erreicht hat (causaler Gesichtspunkt).

5. Schneidet man den Schwanz schräg ab, so stehen die Flossenstrahlen in dem neuen Theil zuerst unter rechten Winkeln zur Schnittfläche. Ein ähnliches Resultat ist bei mehreren anderen Thieren beobachtet worden. Die Auslegung dieses Verhältnisses ist im Text erörtert und der Schluss gezogen, dass er durch die Entwicklung eines symmetrischen Aufbaues des neuen Theils entsteht, wenn die erste Differenzirung beginnt.

6. Die Brust-, Becken-, Rücken-, Schwanz- und Afterflossen von *Fundulus* besitzen Regenerationsvermögen.